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Differentiation of Intestinal Cells In Vitro

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I. INTESTINAL EPITHELIUM: A MODEL SYSTEM FOR STUDY OF CELL DIFFERENTIATION AND POLARIZED CELL FUNCTIONS

The intestinal epithelium is a valuable tissue for study of *cell differentiation* in that it is spatially organized around its proliferative units, the crypts. Each crypt consists of a clone of cells [Ponder et al. 1985] produced by a group of undifferentiated, proliferative cells whose progeny express at least four dramatically different phenotypes [Leblond and Cheng, 1976]. The determinants of phenotype commitment and expression in this tissue are unknown. The temporal sequence of differentiative changes occurring in each cell type can be readily defined, however, since the orderly upward migration of cells from crypt to villus arranges the cells along this axis in order of age [Madara and Trier, 1987]. A major unfulfilled goal of intestinal culture systems is the controlled recreation of the crypt-villus axis of epithelial differentiation in the absence of the complex connective tissue lamina propria.

Like all simple epithelia, the intestine provides a valuable model for study of *cell polarity*. Fully differentiated intestinal cells are more strictly polarized than many other epithelial cell types, perhaps because they are designed to face the threats of the intestinal lumen, a situation in which mis-sorting of membrane components could compromise the epithelial barrier. Also unique to this epithelium is the dramatic conversion that occurs during fetal development from a nonpolarized, stratified cell layer to a simple columnar epithelium [Trier and Moxey, 1979; Colony and Neutra, 1983]. This process provides the opportunity to follow normal, in vivo assembly of tight junctions and de novo formation of apical membrane domains [Madara et al., 1981].

The specialized apical domain of intestinal absorptive cells provides a valuable system for study of the *synthesis and processing of membrane glycoproteins*. The microvillus membrane is rich in intramembrane transporters, membrane-anchored hydrolases, and other glycoproteins, many with characteristic patterns of intracellular and cell surface processing [Hauri, 1983; Semenza, 1986]. Indeed, expression of specific microvillar hydrolases is currently the most reliable indicator of intestinal cell differentiation in vitro. Errors in processing and transport have been identified and associated with clinical malabsorption in humans, and differentiated cell culture systems have recently been used for accurate analysis of these molecular processes [Hauri et al., 1985].

The apical brush border of intestinal absorptive cells, designed to expand the membrane surface area available for hydrolysis and transport, provides a unique model for study of *cytoskeleton assembly* and *cytoskeleton–membrane* interactions. The molecular architecture of this structure has been defined in considerable detail using normal intestinal cells [Mooseker, 1985] but synchronously differentiating in vitro systems provide an opportunity to define

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the factors that control assembly and maintenance of this unique polarized structure.

The intestinal epithelium conducts both absorption and secretion of ions and water. Because it is an extensive and accessible epithelial surface, its *ion transport and permeability* characteristics have been defined in considerable detail [Powell, 1987]. Extracellular and intracellular mechanisms that control ion movements across this monolayer are complex, however, and vary with both cell type and stage of differentiation [Marcial and Madara, 1984]. Such variations have been partially defined using intact mucosal tissue, but the availability of well defined monolayer cultures opens the way for rapid advances in understanding transport phenomena.

Intestinal absorptive cells also provide unique models for study of *endocytosis and transepithelial transport*. In suckling rodents, sorting of IgG–receptor complexes from soluble proteins, and selective transepithelial transport, occurs in jejunal absorptive cells [Rodewald and Abrahamson, 1982]. Ileal cells in sucklings contain a highly polarized system of endosomal compartments that have been isolated and found to contain a membrane antigen that serves as a useful marker for ileal cell endosomal tubules [Wilson et al., 1987a]. In adults, intestinal absorptive cells conduct receptor-mediated transport of polymeric immunoglobulins from basolateral to apical surfaces and express for this purpose a membrane receptor with a unique itinerary (Golgi—> basolateral—>apical) that is cleaved and released into the lumen along with its ligand [Mostov et al., 1980; Mostov and Blobel 1983]. In addition, unique epithelial "M" cells in lymphoid follicle-associated epithelium are models for study of transepithelial transport in that they are highly endocytic but seem to lack a lysosome-directed pathway, instead directing all endocytic vesicles to a specialized basolateral surface [Neutra et al., 1987].

Finally, the mucin-secreting goblet cells of the intestinal epithelium are valuable models in which to study the process of *regulated secretion*. Their large, clearly polarized Golgi complex has facilitated localization of enzymes involved in O-linked glycosylation [Roth, 1987]. The relatively slow, highly polarized movement of mucin secretory granules in goblet cells and the dramatic structural events that accompany stimulated secretion make them a potentially valuable model for study of polarized secretory cell function [Neutra and Forstner, 1987]. They have not been fully exploited for this purpose, however, because normal goblet cells are a minority in the complex and heterogeneous intestinal mucosa and have not been available for study in isolation.

The goal in establishing intestinal cell culture systems is to obtain cells and tissues that mimic as closely as possible their counterparts in vivo. Ideally, epithelial cell cultures should allow the process of normal cell differentiation and function or neoplastic transformation to be recapitulated, manipulated, and observed under controlled conditions. This has proved particularly diffi-

cult to achieve for the intestinal epithelium, where the normal in vivo state is a complex, dynamic one, with continuous cell proliferation and cell loss, in which individual cells survive only for a few days. It is thus useful to review the salient features of intestinal differentiation in vivo as a reference point for assessing available in vitro systems.

II. INTESTINAL EPITHELIAL CELL DIFFERENTIATION IN VIVO

A. Adult: The Crypt-Villus Axis

The intestinal epithelium is a vast sheet of cells composed of millions of tiny differentiating units. Stem cells located near the bottom of each crypt give rise to proliferative cells whose progeny differentiate along one of several paths and migrate slowly up the crypt wall, with the exception of Paneth cells that remain at the crypt base [Leblond and Cheng, 1976]. The three major migratory cell types, columnar cells (enterocytes), goblet cells, and entero-endocrine cells, acquire differentiated features and perform important physiologic functions while still in the crypts. For example, crypt enterocytes actively secrete chloride ions [Welsh et al., 1982] and conduct receptor-mediated transepithelial transport of secretory immunoglobulins from the lamina propria into the lumen [Mostov and Blobel, 1982]; crypt goblet cells conduct acetycholine-regulated mucin secretion [Specian and Neutra, 1980; Neutra and Forstner 1987]; and crypt endocrine cells release regulatory peptides and amines in response to neural, endocrine, and luminal signals [Walsh, 1987]. As these cells migrate out of the crypts and onto the villi (in the small intestine) or onto the flat mucosal surface (in the colon, appendix, and rectum), they rapidly undergo a second phase of differentiation. At the crypt mouth, columnar cells assemble highly organized apical microvillous borders [Madara and Trier, 1987] and express membrane-associated enzymes for terminal digestion and absorption of nutrients [Moog 1979; Simon et al., 1979]. At the same transition point, goblet cells lose cholinergic sensitivity [Specian and Neutra, 1980; Phillips et al., 1984]. Because of continuous cell production, movement of new cells up the crypt wall and cell loss from the villus tip, this entire sequence of differentiative events is displayed along each crypt-villus axis at any moment in time (Table I). This has permitted relatively precise definition not only of morphologic and enzymatic changes but also of the appearance and disappearance of antigenic markers during intestinal cell differentiation in vivo [Quaroni, 1984; Quaroni and Isselbacher, 1985].

B. Rat Fetal and Neonatal Development

Prior to the establishment of the adult steady-state system, the epithelium as a whole passes through major differentiative stages. These stages have been documented most thoroughly in rats and mice, in which rapid differentiation

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TABLE I. Landmarks of Epithelial Differentiation in Adult Small Intestine: The Crypt-Villus Axis*

	Crypt	Transition	Villus	
Cell position	Base	Mouth	Base	Tip
Morphologic features of enterocytes	Short microvilli free ribosomes	Brush border assembly	Long microvilli terminal web	Cell sloughing
	Occluding junctions simple		Occluding junction complex	
Physiologic parameters	^{3}H-thymidine uptake, mitosis mRNA synthesis			
	Goblet cells: cholinergic sensitivity		Goblet cells: insensitive to cholinergics	
	Secretagogue-induced chloride secretion		Ion-water absorption Na-dependent nutrient transport	
Enzyme activities	Thymidine kinase		Three disaccharidases Four peptidases } Apical	
		Basolateral Na, K-ATPase Apical Alkaline phosphatase		
Other antigens and biochemical markers		28–34 kD antigen	Fucose-rich apical glycoproteins	

*For references, see section IIA in text.

occurs late in fetal life, establishing a highly differentiated epithelial system specialized for the suckling period, the first three weeks of life (Table II). In humans, a similar stage occurs much earlier, during the first trimester of pregnancy, but is maintained only until the midpoint of gestation and is no longer present during the suckling period.

1. Predifferentiated fetal epithelium. The intestinal epithelium of fetal rats 1 week before birth (and of fetal humans at 8–9 weeks gestation) is a multilayer of undifferentiated cells [Trier and Moxey, 1979]. In rats, cells of the upper layer are joined by tight junctions that prevent paracellular passage of macromolecules, and luminal membranes have short, sparse microvilli [Trier and Moxey, 1979; Colony and Neutra, 1985]. Deeper cells show no polarity and mitoses occur throughout the epithelium [Hermos et al., 1971]. At this

TABLE II. Markers of Epithelial Differentiation During Normal Rat Intestinal Development*

Developmental stage	Age of onset	Morphologic indicators	Biochemical and immunological markers
Fetal			
Pre differentiation	Up to 15 days of gestation	Simple columnar epithelium Apical tight junctions	Villin: diffuse cytoplasmic
Proliferation	15–16 days of gestation	Stratified epithelium (mammals)	"Fetal antigens" throughout eptihelium
Epithelial conversion	17–19 days of gestation	Secondary lumens Apical cell loss Simple columnar epithelium reestablished	Alkaline phosphatase pI 7–8 (apical) Na,K-ATPase (basolateral)
Cytodifferentiation Stage I	19–21 days of gestation	Assembly of brush borders Villus formation	Villin localized in brush borders 110 kD protein localized in brush borders TW 260/240 localized in brush borders
		Assembly of apical endocytic complexes	IgG Fc receptors (proximal) Endosomal gp55–61 (distal)
			Brush border enzymes appear: Alkaline phosphatase (pI 5–6)

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Cytodifferentiation Stage I	19–21 days of gestation	Assembly of brush borders Villus formation	Villin localized in brush borders 110 kD protein localized in brush borders TW 260/240 localized in brush borders
		Assembly of apical endocytic complexes	IgG Fc receptors (proximal) Endosomal gp55–61 (distal)
			Brush border enzymes appear: Alkaline phosphatase (pI 5–6)
			Lactase Aminopeptidase Maltase (low level) Other antigens
		Other cell types appear: Enteroendocrine cells Paneth cells Goblet cells	Enteric peptide hormones Lysozyme Mucin
Establishment of proliferative units	1–5 days after birth	Crypt formation: mitosis confined to crypts	"Fetal antigens" confined to crypts
Cytodifferentiation Stage II	18–25 days after birth	Adult-type absorptive cells (no apical endocytic complexes)	Disappearance of IgG receptors Disappearance of gp55–61 Decrease in lactase
		Villi in proximal colon with small intestinal morphology and enzymes	Brush border enzymes increase: Aminopeptidase Maltase Sucrase-isomaltase appears
		Crypt goblet cells respond to acetylcholine	Other brush border enzymes and antigens appear

*For references, see text.

stage, "fetal antigens" representing carbohydrate epitopes are present throughout the epithelium [Quaroni, 1986a]. These antigens are limited to cells of the crypts and lower villi in suckling rats and to crypt cells alone in adults. Between 17 and 20 days gestation in rats (9–10 weeks in humans), the stratified epithelium is converted to simple columnar by a complex process involving formation of new tight junctions and secondary lumens and sloughing of apical cells into the lumen [Trier and Moxey, 1979; Madara et al., 1981]. During this process, in rats, polarized apical and basolateral "marker" phosphatases (alkaline phosphatase and Na, K-ATPase) first became detectable, already sorted to the proper domain [Colony and Neutra, 1983; Amerongen et al., 1987]. At 18 days in rat small intestine and at 19 days in colon, differentiated goblet and endocrine cells begin to appear, identified by their characteristic storage granules. In general, differentiative changes appear first in proximal intestine and 1–2 days later in distal regions.

2. Cytodifferentiation stage I. About 1 day after the conversion of the epithelium to a monolayer in rat small intestine (19 days) and colon (20 days), a dramatic burst of cytodifferentiation occurs, and a variety of polarized, differentiated features suddenly appear (Table II). For example, the microvilli of absorptive cells elongate and dramatically increase in number while assembly of the complex brush border cytoskeletal morphology is completed. In chickens, in which the epithelium is a simple monolayer throughout development, assembly of the brush border cytoskeleton from previously synthesized proteins is completed just before hatching [Shibayama et al., 1987]. In rats, integral membrane enzymes such as lactase and aminopeptidase are synthesized and inserted into apical microvillus membranes at 17–19 days, and maltase appears about 2 days later [Koldovsky, 1969; Quaroni, 1985a]. At the same time, specialized endocytic membrane systems assemble at the apical poles of absorptive cells, as if in anticipation of birth and the onset of suckling [Trier and Moxey, 1979; Wilson et al., 1987b]. At this stage, regional differences in absorptive cells appear: In proximal small intestine, the membrane systems designed for Fc receptor-mediated uptake of maternal IgG appear, including polarized apical endosomal compartments [Colony and Neutra, 1985]; in distal small intestine and proximal colon, specialized intermicrovillus membrane invaginations and apical endosomal tubules develop bearing nonintegral luminal arrays; this endosomal membrane system contains an integral 55–61 kD glycoprotein that serves as a marker for these membranes throughout the suckling period [Wilson et al., 1987a]. In the 2 days before birth, endosomal vesicles and multivesicular bodies are assembled in the apical cytoplasm of ileal absorptive cells, while dense vesicles, presumably primary lysosomes, accumulate near the Golgi complex [Wilson et al., 1987b]. Luminal macromolecules, in part derived from swallowed amniotic fluid, are endocytosed by both proximal and distal absorptive cells in late fetal life, and

both transepithelial transport and lysosomal digestion occur [Colony and Neutra, 1985].

Relatively few dramatic changes occur at birth in rats. With the first milk meal, multivesicular bodies and lysosomes in ileal absorptive cells rapidly fuse to form the giant lysosomal vacuole in which milk components are digested [Wilson et al., 1987b]. Transepithelial transport of maternal IgG [Rodewald and Abrahamson, 1982] and growth factors present in milk [Siminoski et al., 1986; Gonnella et al., 1987] occurs in proximal and distal absorptive cells, respectively. Mitotic activity, widespread in the fetal epithelium, becomes limited to intervillus regions and then to the crypts that develop during the first few days after birth, establishing the crypt-villus axis of differentiation [Hermos et al., 1971; Quaroni, 1985b]. Three new apical antigens of unknown function appear after birth and disappear at weaning [Quaroni, 1985a]. In general, however, the brush border protein profile, in which identified enzymes account for a minority of membrane proteins [Hauri et al., 1985], is stable from before birth to the end of the suckling period in rats [Quaroni, 1985a]. The relative paucity of brush border hydrolases during the suckling period is related to the importance of intracellular lysosomal digestion of nutrients during this stage.

3. Cytodifferentiation stage II. At weaning (18–25 days postnatally in rats), the final phase of differentiation is accomplished by production of new, adult-type cells from proliferative precursors in the crypts and progressive displacement of suckling-type cells toward the villus tips [Koldovsky, 1969; Moog, 1979]. Thus individual absorptive cells do not change phenotype, but the epithelium as a whole shows dramatic changes from cell replacement (Table II). Brush border membranes of adult villus cells are rich in hydrolytic enzymes; some of those that were present on suckling cells shift to more acidic forms, and new enzymes appear [Quaroni, 1985a]. Endocytic activity declines, and the cells with elaborate apical endocytic systems with their marker antigens, along with IgG receptors in jejunum and gp 55-61 in ileum, disappear. Crypt goblet cells produced during and after weaning are responsive to cholinergic secretagogues, in contrast to goblet cells of fetal and suckling intestine in which cholinergic agents have no effect [Neutra et al., 1984]. After weaning, a stable sequence of cell differentiation along the crypt-villus axis is present and persists through adult life, as described above (Table I).

C. Human Fetal Development

Much less information is available about human fetal intestinal differentiation, but morphologic and tracer studies have established that the human intestine is relatively mature at birth [Grand et al., 1976]. The same general sequence of differentiative changes observed in rats occurs early during human fetal life [Trier and Moxey, 1979]. At 8–9 weeks of gestation, the human epithelium is stratified. Goblet cells appear in the stratified epithelium as early as 9–10 weeks

and endocrine and Paneth cells soon thereafter. Formation of secondary lumens, conversion to simple columnar epithelium, and formation of villi occurs by 10–11 weeks in the duodenum; both villi and crypts are well developed by 12 weeks.

Stage I cytodifferentiation in humans occurs at 10–12 weeks of gestation. Between 10 and 20 weeks, absorptive cells that line villi throughout the human fetal intestine bear morphologic resemblance to cells of suckling rat ileum, with abundant apical tubules bearing luminal arrays and multiple large lysosome-like vesicles [Trier and Moxey, 1979]. Electron-dense tracers injected into amniotic fluid of a fetal monkey in vivo or injected into the intestinal lumen in an aborted human fetus were endocytosed at this stage but not transported transepithelially [Lev and Orlic, 1973; Moxey and Trier, 1979]. It is not known whether human fetal cells that are actively endocytic express IgG receptors or other components found in suckling rat endocytic pathways or whether they conduct selective transepithelial transport of antibodies, hormones, or other proteins from swallowed amniotic fluid.

Stage II cytodifferentiation in humans occurs at the midpoint of gestation. By 22 weeks, endocytic cells have disappeared in proximal intestine; the exact time of their disappearance from distal regions is unknown [Trier and Moxey, 1979]. Thus an adult-like epithelium is established in the small intestine by the midpoint of human pregnancy, and no dramatic changes occur at birth or at weaning. The human fetal colon at 3–5 months of gestation resembles small intestine in that rudimentary villi are present, and brush border hydrolases; including sucrase-isomaltase and aminopeptidase, are expressed [Koldovsky, 1969; Grand et al., 1976]. These enzymes are absent from colonic epithelial cells at birth and thereafter but reappear in certain well differentiated lines of colon carcinoma cells (see below).

III. INTESTINAL EPITHELIAL CULTURE SYSTEMS

In attempts to reproduce these complex stages of epithelial differentiation, investigators have used three general strategies: 1) separation and culture of normal epithelial cells, 2) maintenance of intestinal explants in organ culture, and 3) culture of neoplastic epithelial cells derived from intestinal adenocarcinomas.

A. Culture of Normal Intestinal Cells

Attempts at establishing primary, differentiated cell cultures or short-term differentiated monolayers from adult intestine have been uniformly unsuccessful. Dispersed epithelial cells are readily obtained from intestinal mucosa of adult rodents, rabbits, chickens, and humans, but they remain viable in culture for only a few hours at most. Such cells have been useful for short-term physiologic experiments that do not require maintenance of polarity, but they

do not proliferate or reestablish monolayers [Moyer, 1983]. This presumably is due to the fact that most such cells are derived from villi and even in vivo would be nonproliferative and short-lived.

As one alternative, intact sheets of epithelium in which tight junctions and crypt-villus architecture are preserved have been obtained from rodents by brief intravascular EDTA perfusion [Bjerknes and Cheng, 1981]. Protein and glycoprotein synthesis continues, apical-basolateral polarity is maintained, and exocytosis can be induced for up to 1 hour in such floating epithelia [Phillips et al., 1984], but basolateral surfaces rapidly lose their normal organization. For example, Na, K-ATPase, normally concentrated in the lateral domain in intact epithelium, moves into the basal domain and is endocytosed shortly after epithelial isolation [Amerongen et al., 1987]. Thus such preparations are of limited usefulness for studies of transport physiology or membrane polarity. It is possible that viability of epithelial sheets could be extended somewhat by culture in or upon media containing appropriate extracellular matrix (ECM) components [Sugrue and Hay, 1982], but it is unlikely that the complex crypt-villus system can be maintained for many hours in this way.

Successful long-term primary culture of undifferentiated intestinal epithelial cells was achieved using benign human tumor cells [Friedman et al., 1981], rat fetal epithelial cells [Negrel et al., 1983], and collagenase-dissociated cells from suckling rat small intestine [Quaroni et al., 1979; Quaroni and May, 1980]. Selected epithelial colonies from the latter cultures were serially passaged, providing nonneoplastic, proliferative lines of intestinal epithelial cells (IEC). These lines, now maintained for nearly a decade, can form monolayers of cuboidal, polarized cells but consistently fail to differentiate in monolayer culture or to express brush border enzymes. Since IEC cells retain proliferative capacity, they presumably are analagous to crypt cells [Quaroni and May, 1980]. Recently, Kedinger and collaborators [1986a, b] succeeded in inducing IEC and fetal cell differentiation by seeding them onto denuded 14 day rat fetal mesenchyme and grafting the recombinant tissue under the kidney capsule of adult rats. Within 10 days, implants that survived developed typical intestinal mucosal structures, with all four epithelial cell types including absorptive cells that expressed typical brush border enzymes. This work established the pluripotent nature of IEC cells and also underscored the importance of mesenchymal cells and their products in intestinal morphogenesis and cytodifferentiation [Kedinger et al., 1981, 1986a, 1987; Haffen et al., 1987]. The recombination grafting technique results in a complex tissue, however, in which individual cell types are as inaccessible as in normal intestine.

B. Culture of Intestinal Explants

Small (2–4 mm^2) samples of intact intestinal mucosa, obtained from humans by biopsy and from experimental animals by surgery, have been maintained in

vitro for up to 24 hours [Trier, 1976; Neutra, 1980; Shields et al., 1979; Arsenault and Menard, 1984]. These preparations have been useful for short-term physiologic studies but are too short-lived for experimental manipulation of cell differentiation. Rodent and chick fetal explants consisting of short tubular segments of intestine, however, can survive for long periods immersed in culture medium and can proceed to differentiate as in vivo [DeRitis et al., 1975; Black and Moog, 1978; Ishizuya-Oka, 1983; Truding et al., 1981; Kondo et al., 1984]. Differentiation of fetal segments also has been demonstrated after subcutaneous implantation into adult rats [Leapman et al., 1974; Montgomery et al., 1981]. In all these cases, survival and differentiation in culture could be initiated only with predifferentiated intestinal segments (17–18 days gestation in the rat) when the epithelium is stratified and cytodifferentiation has not yet begun.

Several attempts have been made to obtain cultured epithelial monolayers derived from undifferentiated fetal intestinal cells. Montgomery and coworkers [1983] cultured mixed populations of trypsin-dissociated intestinal cells from 18 day fetal rats. After initial growth in vitro, cells were injected subcutaneously or intraperitoneally into adult rats, and a differentiated intestinal mucosa was assembled in these sites [Montgomery et al., 1983]. To obtain a more accessible system, mixed fetal epithelial and mesenchymal cells were seeded on collagen substrates or within a collagen "sandwich," with or without added Matrigel, producing villus-like structures on the flat substrates and tubular structures in the sandwich [Montgomery, 1986]. The resulting cultures in all cases consisted of mesenchymal cells underlying a cuboidal or columnar, well-polarized epithelial monolayer with goblet cells, showing proliferative activity. Apical alkaline phosphatase was expressed, but terminal differentiation analagous to villus cells in vivo was not observed [Montgomery, 1986].

Terminal differentiation did occur in epithelial-mesenchymal structures produced by Quaroni [1985c] as outgrowths from 18 day fetal segments in organ culture. Epithelial cells in these outgrowths were tall columnar and highly polarized with multiple cell types, well developed apical brush borders, and expression of many of the plasma membrane antigens present on normal villus cells. In addition, the endocytic complex typical of suckling rat ileal absorptive cells appeared in cells of the cultured outgrowths. Pure epithelial cell cultures isolated from these outgrowths, however, failed to differentiate in culture and resembled the IEC cells derived from suckling rat intestinal crypts described above [Quaroni, 1985c]. Thus, in all systems tested so far, cells derived from normal intestinal epithelium seem to require the presence and presumably the products of intestinal mesenchymal cells in order to differentiate [Haffen et al., 1987; Kedinger et al., 1987]. Standard basal lamina components are present under the normal intestinal epithelium [Laurie et al., 1982] but their exact roles in epithelial differentiation have not been defined. Faced

with this limitation of normal intestinal cell culture, many investigators have turned to immortal cell lines derived from human colonic tumors.

C. Intestinal Adenocarcinoma Cell Lines

Dozens of human adenocarcinoma cell lines are available because adenocarcinoma of the colon is a common human malignancy [Fogh and Trempe, 1975]. The growth properties of many lines in culture and in transplants have been described and reviewed [Fogh et al., 1977]. An antibody that specifically recognized a component present only on normal rat fetal and neonatal intestinal cells also recognized its antigen on 11 of 12 malignant human cell lines [Quaroni, 1986b], confirming the general similarity of neoplastic colonic cells and fetal epithelial cells [Zweibaum et al., 1983, 1984]. Such a relationship had been initially suggested 25 years ago with the discovery of carcinoembryonic antigen [Gold and Freedman, 1965]. Colon carcinomas vary widely in their degree of differentiation in situ and in their differentiative capacity when grown as tumors in nude mice [Fogh et al., 1977]. Differentiation was observed when colon carcinoma cells were cultured on fetal rat mesenchyme [Fukamachi et al., 1986]. (For a detailed review of the metabolic and physiologic properties of established adenocarcinoma cell lines in culture, see Zweibaum et al. [1988] and Chantret et al. [1988].) Under standard cell culture conditions, most cell lines do not differentiate.

1. Caco-2. One important exception is the Caco-2 cell line. Caco-2 cells were derived from a relatively well differentiated tumor and grow slowly in nude mice. When seeded either on permeable filters or impermeable substrates (plastic or glass) at high density, they consistently form well polarized monolayers joined by tight junctions, with well developed apical microvilli [Pinto et al., 1983]. Such monolayers survive for up to 30 days in culture before spontaneously detaching from the substrate. Although derived from adult human colon, where microvillar hydrolases are not expressed, Caco-2 cells express two disaccharidases and two peptidases typical of normal small intestinal villus cells; they also transport ions and water toward the basolateral surface, forming domes on impermeable substrates [Pinto et al., 1983]. Despite this superficial resemblance to normal small intestinal absorptive enterocytes, Caco-2 cells are more closely analagous to enterocytes of the normal 15-week human fetal colon, in which microvillar hydrolases are transiently expressed [Koldovsky, 1969; Grand et al., 1976]. Indeed, Caco-2 membrane hydrolases were shown to occur in molecular forms typical of fetal tissues [Hauri et al., 1985]. In their electrical parameters, ion conductance, and permeability properties, Caco-2 monolayers resemble colonic crypt cells [Grasset et al., 1984]. Whereas crypts contain multiple cell types, however, Caco-2 monolayers are remarkably homogeneous, suggesting that these cells represent the neoplastic equivalent of crypt

enterocytes committed to the absorptive cell line but arrested in differentiation at the crypt/fetal stage.

2. T84. The T84 cell line, originally derived from a lung metastasis of a human colon carcinoma, resembles Caco-2 in its ability to form spontaneously well polarized monolayers of high electrical resistance [Dharmsathaphorn et al., 1984, 1985]. Unlike Caco-2 cells, T84 cells do not form well developed brush borders and fail to express microvillar membrane hydrolases. In their morphology, electrical parameters, and ion transport activities, T84 monolayers resemble adult colonic crypt cells [Madara and Dharmsathaphorn, 1985]. Like Caco-2 cells, T84 cell monolayers are homogeneous and appear to represent a line derived from committed crypt cells (see elsewhere in this volume). In both Caco-2 and T84 cells, monolayer formation and differentiation are accelerated on substrates such as collagen that enhance cell attachment, and differentiation is preceded by synthesis and polarized secretion of basal lamina components [Madara et al., 1987].

3. HT29. The HT29 cell line, in contrast, does not show polarity or other differentiated characteristics of intestinal cells under standard conditions (in media containing glucose and normal serum), and until recently these cells were useful only for studies of general cell features, such as ubiquitous receptors and carbohydrate metabolism [Rousset et al., 1981; Rousset, 1986]. When grown in the absence of glucose, however, HT29 cells exhibit a high degree of differentiation [Pinto et al., 1982], surpassing in some respects all other neoplastic lines developed to date [Rousset, 1986]. Phenotypes resembling terminally differentiated goblet and absorptive cells appeared in highly polarized, confluent HT29 cell monolayers when galactose, inosine, or uridine were substituted for glucose as carbon sources [Pinto et al., 1982; Wice et al., 1985] or even in the total absence of these additives [Zweibaum et al., 1985]. Conditions permissive for differentiation also include the presence of supplemental human transferrin and nonessential amino acids and seeding at high density so that confluence is reached within a few days after plating. Differentiated HT29 cells express four brush border enzymes: alkaline phosphatase, sucrase-isomaltase, aminopeptidase N and dipeptidylpeptidase IV [Pinto et al., 1982; Zweibaum et al., 1983, 1984, 1985], all of which are typical of normal fetal colon. HT29 cells grown in the absense of glucose form highly polarized, differentiated monolayers either on permeable filters or uncoated, impermeable glass. Although they do not secrete a basal lamina visible by electron microscopy, both undifferentiated and differentiated HT29 cells do secrete immunoreactive laminin. Since differentiation can be manipulated in culture, HT29 monolayers offer unique opportunities to reproduce and dissect the process of intestinal cell differentiation and to identify factors that govern the physiologic functions of terminally differentiated intestinal cells.

The value of the HT29 cell line has been further extended by isolation of

clones and subclones. A clone designated HT29-18 was shown to be multipotent, giving rise to both goblet and absorptive cells when grown in the absence of glucose, and a fraction of these cells retained partially differentiated characteristics, such as the ability to form monolayers when returned to glucose-containing medium [Huet et al., 1987]. Subcloning of differentiated HT29-18 cells by limiting dilution and replating in medium containing glucose produced colonies that expressed a single phenotype, either goblet cell or absorptive cell (Fig. 1) [Huet et al., 1987]. Further studies have established that fully differentiated HT29 subclones, like the HT29 parent and cloned lines, show many characteristics typical of terminally differentiated surface epithelial cells in the human fetal colon. These features are described in more detail below.

IV. USE OF IN VITRO SYSTEMS FOR STUDY OF INTESTINAL CELL BIOLOGY

A. Cell Differentiation

IEC cells consistently fail to differentiate in monolayer culture [Quaroni and May, 1980], and so far this requirement has not been fulfilled by coating culture surfaces with ECM components reconstituted from tissue extracts. IEC cells did differentiate when seeded onto denuded native fetal mesenchyme and implanted in vivo (as did fetal endodermal cells); differentiation was induced in these recombinant tissues regardless of the source of the mesenchyme—either intestine or skin [Kedinger et al., 1986b, 1987; Haffen et al., 1987]. Similarly, differentiation of epithelial cells from dissociated fetal tissue [Montgomery, 1986] and in outgrowths of fetal intestinal explants [Quaroni, 1985c] has been obtained only in the presence of mesenchymal cells, although the overall shape of the resulting tissue can be influenced by the contour of the substrate provided [Montgomery, 1986]. Direct epithelial cell interactions with mesenchymal cells occurs during normal fetal development [Mathan et al., 1972; Burgess, 1976] and in vitro this poorly defined interrelationship is a prerequisite for the epithelial differentiative response to glucocorticoids [Kedinger et al., 1987]. Seeding of IEC or fetal endoderm cells onto preformed, cell-free adult intestinal basal lamina or other native matrix has not yet been reported. It is clear, however, that the crypt-villus axis of differentiation is not readily reproduced using normal intestinal cells on the artificial basal lamina preparations now available. In vivo, adult intestinal cells are accompanied during crypt-villus migration by a distinct population of subepithelial fibroblasts that migrates in parallel [Marsh and Trier, 1974; Parker et al., 1974], and these may supply chemical signals that influence expression of fully differentiated epithelial features [Haffen et al., 1987].

The neoplastic cells described above are apparently released from this con-

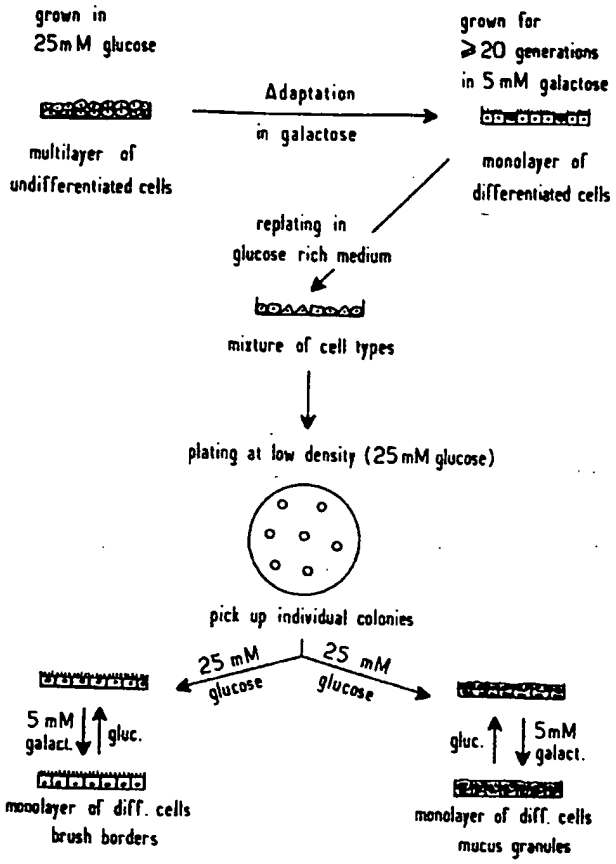


Fig. 1. Schematic diagram showing the method of isolation of HT29 clones and subclones. Clone HT29-18 was isolated after three successive clonings of the parent HT29 cell line. HT29-18 cells adapted to glucose-free medium differentiated into both absorptive and goblet cells. Further cloning of HT29-18 cells yielded subclones that were selected for their ability to differentiate into homogeneous monolayers of either absorptive cells (HT29-18/Cl) or goblet cells (HT29-18/N2). (Reproduced from the Journal of Cell Biology, 1987, 105:349, by copyright permission of the Rockefeller University Press.)

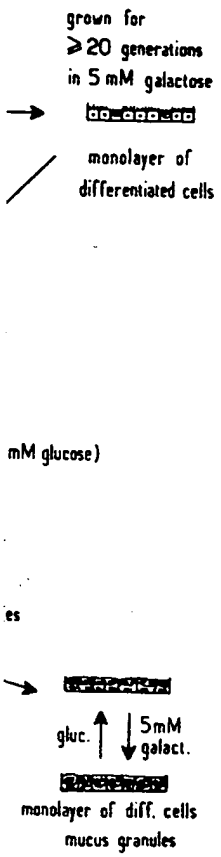
straint and proceed to differentiate without strict substrate requirements. T84 cells in conventional media and HT29 cells in media lacking glucose proliferate and form mixed monolayers/multilayers soon after seeding. Subsequent homogeneous monolayer formation is accompanied by appearance of intracellular and intraepithelial lumens containing apical membrane markers and microvilli [Madara et al., 1987; LeBivic et al., 1988b; Phillips et al., 1988] reminiscent of the secondary lumens that appear in normal fetal intestine during epithelial conversion from stratified to simple columnar [Madara et al., 1981; Colony and Neutra 1983]. This phenomenon may be useful in understanding establishment of membrane polarity, as described below.

Soon after polarized monolayers are established, all three cell lines resemble undifferentiated crypt cells morphologically, but they are not identical. Caco-2 cells seem committed to a single hybrid phenotype, expressing the ion transport properties of colonic crypt cells [Grasset et al., 1984] and some (but not all) of the apical membrane enzymes of fetal colonic villus cells [Hauri et al., 1985]. T84 cells are also committed to the enterocyte phenotype but differentiate only to the level of normal crypt epithelium [Madara and Dharmsathaphorn, 1985; Madara et al., 1987], conducting electrogenic chloride secretion that is induced by secretagogues [Dharmsathaphorn et al., 1984, 1985]. Whether T84 cells grown under other culture conditions could proceed to become analagous to colonic surface absorptive cells is unknown.

HT29 cells mimic more closely the normal crypt-villus system [Zweibaum et al., 1988]. Whereas both the parent line and clone HT29-18 are multipotent, like crypt stem cells, the subclones derived from HT29-18 when immature (shortly after confluence) are analogous to crypt cells committed to a single phenotype, either absorptive cell (HT29-18C1) or goblet cell (HT29-18N2) [Huet et al., 1987]. Other HT29 clones have also been identified as single-phenotype [Augeron and Laboisse, 1984]. Commitment of the parent cells was apparently induced either by forcing an alteration in carbohydrate metabolism [Zweibaum et al., 1985] or by long-term treatment with sodium butyrate [Augeron and Laboisse, 1984]. Evidence from both Caco-2 and HT29 cells indicates that important changes in glucose metabolism accompany differentiation: Glucose consumption and lactic acid production, as well as available UDP-N-acetylhexosamines, decline as glycoprotein synthesis increases. Availability of committed homogeneous clones and subclones has opened the way for study of the differentiation process of single cell types on the crypt-villus axis. Specific studies of HT29 absorptive cells [Dudouet et al., 1987] and goblet cells [Phillips et al., 1988] are described below.

B. Membrane Polarity

Various types of epithelial cells seem to differ in their degree of membrane polarity and their ability to sort apical and basolateral proteins. Some of these



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differences reflect the greater polarity achieved by normal cells in vivo as compared to cultured monolayers, and others may be attributed to the specific properties in vitro of the various cell lines used as models. Polarized delivery of viral glycoproteins in infected MDCK cells requires cell–substrate or cell–cell contact [Rodriguez-Boulan 1983a, 1983b]. Correct insertion of MDCK cell apical membrane components requires lateral cell-cell contact [Vegas-Salas et al., 1987], and maintenance of polarity depends on the tight junctional barrier [Gumbiner and Simons, 1986]. Cells of polarized, tight MDCK monolayers sort endogenous membrane proteins intracellularly in the trans-Golgi compartment [Matlin and Simons, 1984; Fuller et al., 1985]. A transfected membrane protein, the IgA receptor, was also properly sorted by MDCK monolayers [Mostov and Deitcher, 1986]. On the other hand, transfected MDCK cells expressing exogenous secretory proteins released them by "default" onto both surfaces [Gottlieb et al., 1986; Kondor Koch et al., 1985]. Some exogenous secretory proteins expressed in transfected endocrine cells were sorted properly in the trans-Golgi, entering regulated secretory granules [Moore and Kelly, 1985). In part, this may reflect the absence of a regulated secretory pathway in MDCK cells. It is not yet established whether the inability of some transfected cells to sort certain exogenous proteins properly is due to lack of the specialized recognition machinery that correctly sorts these molecules in the cells that normally express them. Terminally differentiated intestinal cells, unlike MDCK cells, show extreme structural polarity and elaborately-organized apical domains [Reggio et al., 1988]. Most of the specialized apical membrane components of enterocytes are not even synthesized until well after a high degree of polarity is established. The site of sorting in intestinal cells, however, is not clearly established. Indeed, the exact itinerary of apical and basolateral membrane proteins from the trans-Golgi cisternae to the plasma membrane in various specialized, differentiated cell types is still controversial, as discussed below.

Cultured monolayers derived from normal rat intestinal cells such as IEC lines have been of limited use in membrane polarity studies, because in monolayer culture they fail to achieve high polarity and do not express polarized membrane markers. Neoplastic intestinal cell lines, in contrast, can achieve high polarity in monolayer culture and express multiple, specific endogenous markers for both apical and basolateral domains (Figs. 2 and 3). Endogenous intestinal cell basolateral membrane components are synthesized and correctly delivered to the basolateral domain in polarized HT29 cell monolayers, including NA, K-ATPase, transferrin receptor, polymeric immunoglobulin (IgA) receptor, and histocompatibility antigen (HLA-1). An antigen of unknown function defined by monoclonal antibodies is also localized in the basolateral membrane of HT29 cells [LeBivic et al., 1988b]. In nonpolarized, undifferentiated

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Fig. 2. Confluent HT29-18/Cl cells grown in medium containing glucose. This absorptive cell subclone forms polarized monolayers with tight junctions and expresses some brush border hydrolases in the presence of glucose. Bar = 1 μm.

HT29 cells grown in glucose-containing medium, these proteins are expressed over the entire surface, but, after formation of apical domains in polarized monolayers grown without glucose, they are strictly confined to the basolateral side [Godefroy et al., 1988]. This phenomenon resembles that observed during cell polarization in normal fetal rat intestine: Na, K-ATPase is distributed uniformly on surfaces of unpolarized cells in the stratified fetal epithelium but is consistently excluded from newly formed apical domains as tight junctions assemble and polarization develops [Colony and Neutra, 1983; Amerongen et al., 1987].

Fig. 3. Confluent HT29-18/Cl cells grown in medium lacking glucose but containing 5 mM galactose. In the absence of glucose, well developed brush borders are formed and the apical membrane contains sucrase-isomaltase (visualized by immunoperoxidase labeling), a marker for terminal differentiation. Bar = 1 μm.

In Caco-2 cell monolayers grown on permeable filters, secretion of the endogenous enterocyte product apolipoprotein (which in adults in vivo would be released into the circulation) and α-fetoprotein (a product of fetal endodermal tissues) occurred only toward the basolateral side as expected [Rindler and Traber, 1988]. Treatment of MDCK cell monolayers with weak bases was shown to disrupt polarized basolateral secretion of laminin, the endogenous product [Caplan et al., 1987], but in Caco-2 cells [Rindler and Traber, 1988] and HT29 cells correct basolateral delivery of endogenous products was not affected by such treatment. Furthermore, secretion of exogenous proteins (human growth hormone introduced by gene transfection and a lysosomal enzyme deficient in glycosylation) was exclusively basolateral in Caco-2 cells [Rindler and Traber, 1988]. Together, these findings indicate that basolateral delivery of membrane proteins and secretory products in intestinal cells could occur partly or entirely by default and may not require sorting signals. In addition, they underscore the danger in extrapolating data obtained using MDCK cells to specialized, highly polarized epithelial cells such as intestinal enterocytes.

Delivery of newly synthesized membrane glycoproteins to the apical domain, in contrast, seems to require the presence of tight junctions [LeBivic et al., 1988b] and functional microtubules [Blok et al., 1981a; Bennett et al., 1984] and probably depends on specific molecular sorting signals. The earliest-detected apical protein expressed in the stratified epithelium of fetal rat colon, alkaline

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phosphatase, appears only after tight junctions have assembled and have defined the apical membrane domains of the mucosal surface and intra epithelial lumens [Colony and Neutra, 1983; Amerongen et al., 1987]. Cells in the fetus that lack apical domains do not express apical antigens. A 170 kD antigen normally confined to human colonic cell apical membranes is expressed in neoplastic HT29 cells even before monolayer formation, but in unpolarized HT29 cells this antigen is weakly expressed and is confined to intracellular vacuoles [LeBivic et al., 1988b]. Undifferentiated HT29 cells grown in glucose have no immunologically detectable sucrase-isomaltase, but metabolic labeling studies revealed that high-mannose and complex forms of the enzyme are synthesized and rapidly degraded. This contrasts with differentiated cells where the enzyme is processed, delivered to the cell surface, and remains stable for at least 48 hours [Trugnan et al., 1987]. Further studies demonstrated that N-glycan processing is severely altered in undifferentiated, neoplastic enterocytes [Ogier-Denis et al., 1988].

Formation of monolayers by T84 cells, as well as HT29 cells grown without glucose, is often accompanied by appearance of intraepithelial lumens defined by junctional complexes and lined by microvilli [LeBivic et al., 1988b; Madara et al., 1987; Phillips et al., 1988]. During monolayer formation in HT29 cells, the apical 170 kD antigen appeared on these small lumens as well as on the apical side of the epithelium once tight junctions were present [LeBivic et al., 1988b]. Formation of aberrant lumens between or within differentiating HT29 or T84 cells is thus reminiscent of normal fetal events. Although it must be recalled that these are neoplastic cells, the mechanisms that direct apical membrane insertion in normal cells are presumably operative in neoplastic cells as well. Thus these culture systems permit de novo formation of apical membrane to be followed (and in HT29 cells to be manipulated with culture conditions) so that factors controlling expression and sorting of apical membrane glycoproteins can be defined more clearly.

There is evidence that sorting of apical glycoproteins in intestinal cells, as in MDCK cells, occurs in the trans-Golgi compartment based on the distribution of these proteins in subcellular fractions after metabolic labeling [Danielson and Cowell, 1985] and on immunolocalization of intestinal apical proteins [Fransen et al., 1985]. There is also evidence, however, for initial targeting of apical glycoproteins to the lateral membrane, followed by rapid sorting and selective transepithelial transport to the apical side [Louvard, 1980; Massey et al., 1987]. Recent evidence from metabolic labeling studies in hepatocytes has revealed a similar pattern: Glycoproteins concentrated in the bile canalicular (apical) membrane at steady state appear transiently in the sinusoidal (basolateral) membrane soon after synthesis [Bartles and Hubbard, 1988]. Intestinal cells share with hepatocytes an endodermal origin, constitutive basolateral secretion of certain serum proteins, a basolateral-to-apical transport pathway

for IgA, and a highly specialized apical domain. It is thus conceivable that all intestinal apical membrane glycoproteins could first be inserted basolaterally, along with the IgA receptor, and then rapidly sorted for delivery to the apical domain [Bartles and Hubbard, 1988]. On the other hand, intestinal cells synthesize much larger amounts of apical membrane constituents than do hepatocytes: At least half of newly synthesized, [^{3}H]fucose-labeled membrane macromolecules appeared in apical membrane soon after synthesis, and predominantly basolateral delivery was observed only after disruption of microtubules with drugs [Blok et al., 1981a; Bennett et al., 1984]. Further studies using cultured monolayers of well differentiated intestinal cells should finally resolve this issue.

C. Synthesis and Processing of Apical Membrane Glycoproteins

Glycoproteins of the microvillus membranes show diverse and unique features that have been extensively investigated and reviewed [Hauri, 1983; Semenza, 1986]. Sucrase-isomaltase, for example, is synthesized in mammalian enterocytes as a large precursor that is processed in the rough endoplasmic reticulum (RER) and Golgi, and cleaved into two subunits—the larger intramembrane isomaltase and smaller sucrase—after insertion in the apical membrane by cell-surface proteases derived from pancreatic secretions [Hauri et al., 1982]. In the fetus, where pancreatic protease secretion is negligable, final processing does not occur, and the high-molecular-weight form persists in the apical membrane [Hauri, 1983]. Caco-2 cells express two of the three disaccharidases (sucrase-isomaltase and lactase) identified in microvillar membranes of normal intestine. Metabolic labeling and immunochemical studies of Caco-2 hydrolases revealed that the 217 kD single-chain precursor form of sucrase-isomaltase persists in Caco-2, as in human fetal intestine, and also confirmed that lactase is synthesized as a large precursor that is proteolytically cleaved, perhaps intracellularly, without the participation of pancreatic enzymes [Hauri et al., 1985]. Caco-2 cells also express aminopeptidase N and dipeptidylpeptidase IV in normal fetal forms, slightly smaller than the normal adult human enzymes. Pulse-labeling studies using the Caco-2 monolayer system further revealed that microvillar proteases are transported from RER to Golgi more rapidly than are disaccharidases [Hauri et al., 1985]. Detailed understanding of the synthetic and processing steps involved in normal enzyme production in enterocytes, derived in part from monolayer culture systems, is important for eventually elucidating the molecular and genetic defects underlying human clinical malabsorption syndromes such as lactase and sucrase-isomaltase deficiency [Naim et al., 1988].

D. Development of a Polarized Cytoskeleton

The molecular architecture of the enterocyte brush border has been extensively studied as a model system for membrane-cytoskeleton interactions and assembly of actin filaments and filament bundles [for review, see Mooseker,

1985]. The apical microvilli of enterocytes contain cell-specific actin-associated components, including villin, a 95 kD protein that controls polymerization of actin [Bretscher and Weber, 1980; Mooseker, 1985] and a 110 kD protein–calmodulin complex that contains actin-activated Mg-ATPase activity and forms periodic actin-membrane cross bridges along the microvillus [Collins and Borysenko, 1984; Conzelman and Mooseker, 1987], in association with a 140/200 kD membrane glycoprotein [Coudrier et al., 1983]. Microvillus actin core rootlets also interact with an enterocyte brush border-specific form of spectrin [Glenney and Glenney, 1983; Pearl et al., 1984].

Immunocytochemical studies in developing chick embryos showed that key elements of the brush border cytoskeleton are present in diffuse form early in development, but their assembly in the brush border occurs in stages and is completed just before hatching when typical brush border ultrastructure is established. Three actin-associated proteins of the microvillar core moved into place at the apical pole at different times: villin at 8 days, fimbrin at 10 days, and 110 kD protein only at 21–22 days [Shibayama et al., 1987].

Villin in mammalian enterocytes is also diffusely distributed in undifferentiated cells of adult crypts and fetal intestine but concentrates in the apical brush border as cells undergo terminal differentiation [Robine et al., 1985]. Because villin is present in enterocytes at all differentiative stages, including primitive fetal endoderm, and in tumors derived from intestinal epithelium regardless of their state of differentiation, it provides a diagnostically valuable antigenic marker for identification of adenocarcinomas of intestinal origin [Robine et al., 1985].

Terminal differentiation of HT29 cells, the multipotent clone HT29-18, and the enterocyte subclone HT29-18C1 is accompanied by assembly of well developed brush borders (Fig. 3). The presence and distribution of villin, like other markers of differentiation, was dramatically altered by shifting cells from glucose-containing to glucose-free media. Enzyme-linked immunosorbant assay (ELISA) and immunoflourescence using specific antivillin monoclonal antibodies revealed that villin in well differentiated HT29-18 cells is as abundant as in normal human enterocytes and is concentrated in microvillar cores, whereas villin in undifferentiated HT29-18 cells is diffusely distributed and present at low levels [Dudouet et al., 1987]. HT29-18 cells dramatically increase rates of villin synthesis during differentiation, attributable either to an increase in transcription or to stabilization of villin mRNA [Pringault et al., 1986]. The HT29 cell system, in which brush border assembly can be controlled by manipulating culture conditions, opens the way for clearer understanding of brush border assembly using molecular biological approaches.

E. Vectoral Ion Transport

Net ion and water movements across the normal intestinal epithelium represent the sum of movements across distinct microareas: ion secretion from

crypts and ion absorption from villi [Welsh et al., 1981; Powell, 1987]. Furthermore, fluxes within each area may represent the sum of paracellular permeability differences among various cell types [Marcial et al., 1984]. The homogenous monolayer culture systems T84 and Caco-2 have been proved valuable alternatives to the complex normal situation even though they are derived from neoplastic cells that may differ in some respects from normal enterocytes. Transport and permeability studies using the T84 cell system are described in detail elsewhere in this volume.

Caco-2 cells grown under standard culture conditions show ion transport properties analagous to normal colonic crypt cells and not to cells on the mucosal surface [Grasset et al., 1985]. Differentiation of Caco-2 cells was accompanied by a tenfold increase in receptors for vasoactive intestinal peptide (VIP), a secretagogue that induces vectoral transport of fluid and electrolytes in normal intestinal crypts [Laburthe et al., 1987]. Caco-2 cell monolayers are sensitive to physiological secretagogues such as VIP and other agents that elevate intracellular cyclic AMP concentrations, and respond with an apically directed secretion of chloride ions [Grasset et al., 1985], but they do not exhibit electrogenic, amiloride-sensitive sodium absorption [Grasset et al., 1984]. Caco-2 cells grown on impermeable substrates in the absense of secretagogues form domes, however, indicating their ability to transport ions in an apical to basolateral direction [Pinto et al., 1983]. Caco-2 cell monolayers develop tight junctions that prevent passage of macromolecules [Hidalgo et al., 1988], as do cells of normal crypts [Phillips et al., 1987], and provide electrical resistance of about 150 ohms/cm^2 [Grasset et al., 1984]. This value is somewhat lower than that of normal colonic mucosa in vivo, where both leaky crypt and tighter surface junctions are present [Powell, 1987].

F. Endocytosis and Transport of Macromolecules

Nonselective endocytosis and transepithelial transport of luminal macromolecules occur in the rat fetal intestinal epithelium at all stages [Colony and Neutra, 1985]. In rats, specific receptor-mediated endocytosis and transepithelial transport of milk macromolecules, including maternal IgG [Rodewald and Abrahamson, 1982] and growth factors [Siminoski et al., 1986; Gonnella et al., 1987], is confined to the absorptive cells of the suckling period and involves highly polarized apical endosomal compartments [Wilson et al., 1987a]. The IEC monolayer culture system derived from normal rat intestinal crypts does not produce suckling-type endocytic cells even when fully differentiated on mesenchymal substrates [Kedinger et al., 1986b]. Cultures derived from rat fetal explants including fetal mesenchyme did produce epithelial cells with the apical tubules and lysosomal vacuoles characteristic of the suckling rat ileum, but pure epithelial monolayers isolated from these explants failed to develop such features [Quaroni, 1985].

Adult rat and human absorptive cells nonselectively endocytose small amounts of luminal protein but generally direct them to apical lysosomes [Blok et al., 1981a]. Absorptive cells with apical endocytic systems morphologically comparable to those seen in suckling rat ileum are present in human fetal enterocytes between 10 and 20 weeks of gestation [Trier and Colony, 1979], but such cells have not been observed to date in cultured human adenocarcinoma cell lines. Differentiation of HT29 cells in glucose-free medium, for example, seems to recapitulate the crypt-villus axis of development in fetal colon at a later gestational stage, after the disappearance of endocytic cells. Caco-2 cells are also comparable to fetal colonic cells at a stage that lacks endocytic complexes. Like normal adult rat and human enterocytes, they endocytose fluid-phase tracers from both apical and basolateral surfaces and direct the tracers to apical lysosomes [Hidalgo et al., 1988]. Thus there is presently no monolayer system in which the elaborate, polarized endocytic systems of fetal/neonatal enterocytes are reproduced in monolayer culture.

Adult intestinal cells in the ileum conduct receptor-mediated endocytosis of intrinsic factor (IF)–cobalamin (vitamin B_{12}) complexes from apical coated pits between microvilli [Levine et al., 1986], but the intracellular fates of IF and its receptor are unclear. Recently, Caco-2 cell monolayers were shown to bind and internalize both IF–cobalamin and cobalamin alone, and the receptor was identified as a 90 kD glycoprotein, contrasting with the 180 kD species identified as a cobalamin receptor in normal human ileum [Muthiah and Seetharam, 1987].

The basolateral membrane of adult enterocytes contains receptors for transferrin and polymeric IgA, both of which mediate uptake of their respective ligands into basolateral endosomes [Slot and Geuze, 1984; Banerjee et al., 1986]. In HT29-18C1 cells, as in other polarized cells [Klausner et al., 1983], ferrotransferrin uptake into acidic compartments delivers iron to the cell, and apotransferrin–receptor complexes are recycled to the basolateral plasma membrane [Godefroy et al., 1988]. IgA–receptor complexes, in contrast, do not recycle but are delivered to the apical surface, where the receptor is cleaved, releasing secretory IgA into the lumen [Mostov and Blobel, 1982]. Separation of flourescently labeled IgA and tranferrin after basolateral endocytosis in enterocytes has been reported, but detailed studies on receptor movements in the endocytic membrane systems at the basolateral cell surface are lacking.

The HT29 cell system provides an excellent enterocyte-like model in which basolateral receptors can be followed morphologically and biochemically. In addition to receptors for transferrin, Ia antigen (MHC II) is present on basolateral membranes of normal crypt enterocytes [Gorvel et al., 1984; Mayrhofer, 1984]. Both transferrin receptors and histocompatibility antigen are basolateral on well polarized HT29 cells. These two membrane proteins are uniformly dis-

tributed on nonpolarized HT29 cells but acquire polarized distribution during differentiation, being excluded from the newly formed apical membrane domain [Godefroy et al., 1988]. Study of transferrin receptor, IgA receptor, and other molecular traffic in HT29 cells will serve to extend the information already gained from other polarized cell systems such as hepatocytes. Further study of the synthesis, trafficking, and function of intestinal epithelial histocompatibility complexes in HT29 cells may reveal their exact function in normal intestine, which is presently unknown; this may help to clarify the interactions of crypt enterocytes with luminal antigens and with cells of the mucosal immune system [Mayrhofer, 1984].

G. Regulated Secretion

Much of the information currently available regarding the mechanisms of sorting of secretory proteins into constitutively secreted vesicles or regulated storage granules has been derived from endocrine cells [for review see Kelly, 1985]. Polarized epithelial monolayer culture systems that reproduce the regulated, polarized secretion of exocrine cells have not generally been available. The AR42J rat pancreatic cell line, proposed for this purpose, synthesizes amylase and releases it in response to cholecystokinin when grown in monolayer culture [Logsdon et al., 1984] but these cells do not store secretory granules to the same degree as do normal pancreatic cells.

The HT29-18N2 subclone now provides a valuable alternative. When grown in the absense of glucose, these cells form highly polarized, homogeneous monolayers showing the morphological features of fully differentiated intestinal goblet cells [Huet et al., 1987], a cell type highly specialized for exocrine secretion of mucin [Neutra and Forstner, 1987]. The sequence of morphologic changes in HT29-18N2 cells during cytodifferentiation in confluent monolayers resembles that seen during goblet cell differentiation in normal crypts in vivo [Phillips et al., 1988] (Fig. 4). Immunocytochemical analysis, using a panel of monoclonal antibodies directed against defined oligosaccharide and peptide epitopes of normal human colonic mucin, revealed that HT29-18N2 cells synthesize human colonic mucin but show more heterogeneity in oligosaccharide epitopes than do normal human goblet cells (Fig. 5). Constitutive secretion of glycoproteins was detected prior to differentiation, but immunoreactive mucin appeared only after formation of stored secretory granules. Terminally differentiated HT29-18N2 goblet cells respond to cholinergic secretagogues, analagous to intestinal crypt goblet cells [Phillips et al., 1988].

These cells can be grown on both permeable and impermeable substrates, allowing application of secretagogues to basolateral membranes. In addition, differentiation can be modulated by changing culture conditions so that some degree of synchrony can be obtained, a situation impossible to obtain in vivo. HT29-18N2 goblet cells store secretory granules to the same extent as their

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able alternative. When grown
ly polarized, homogeneous
of fully differentiated intesti-
ghly specialized for exocrine
. The sequence of morpho-
rentiation in confluent mono-
ferentiation in normal crypts
tochemical analysis, using a
defined oligosaccharide and
n, revealed that HT29-18N2
more heterogeneity in oligo-
t cells (Fig. 5). Constitutive
differentiation, but immuno-
f stored secretory granules.
respond to cholinergic secre-
s [Phillips et al., 1988].
nd impermeable substrates,
ral membranes. In addition,
ture conditions so that some
impossible to obtain in vivo.
to the same extent as their

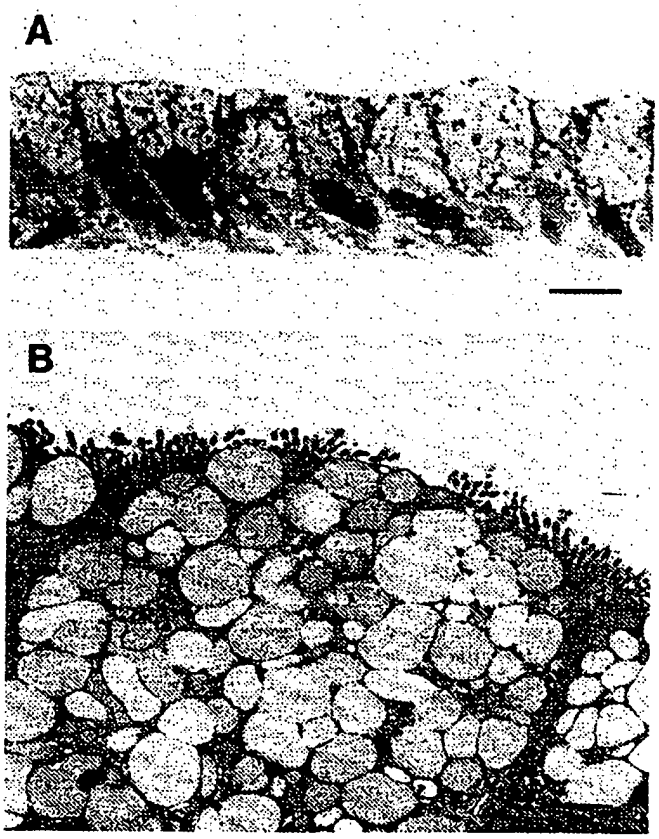

Fig. 4. Confluent HT29-18/N2 cells grown in medium lacking glucose but containing 5 mM galactose. **A:** By light microscopy, this subclone forms a homogeneous monolayer of goblet cells when fully differentiated. Bar = 10 μm. **B:** By electron microscopy, the apical poles of diffentiated cells are filled with large granules typical of mucin-secreting cells. Bar = 1 μm.

counterparts in vivo [Neutra and Forstner, 1987]. This subclone can now be exploited to gain a clearer understanding of some of the key functions common to regulated exocrine cells, including mechanisms of sorting of secretory proteins into regulated granules, assembly of storage granules and their unique membrane components, and mechanisms governing compound exocytosis during stimulated secretion.

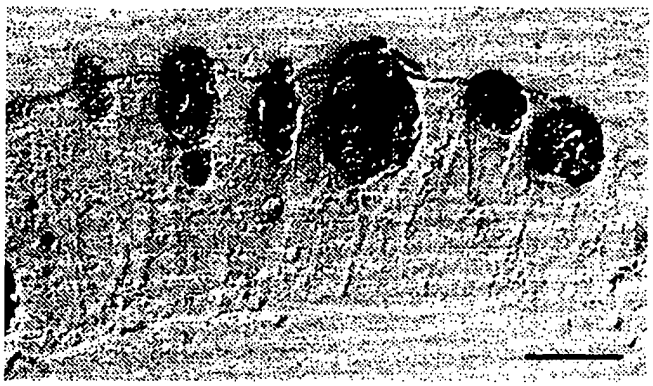

Fig. 5. Nomarski image of confluent HT29-18/N2 cells grown as for Figure 4, but not yet fully differentiated. Differentiation of monolayers is not synchronous: Some cells have many secretory granules containing immunoreactive mucin (visualized by immunoperoxidase labeling); others have not yet begun mucin synthesis or storage. Bar - 10 μm.

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